

## Evaluation of the antidiarrhoeal and antidiabetic activities of the leaf aqueous extract of *Syzygium cordatum* hoscht. ex C.Krauss (Myrtaceae) in rodents

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### Abstract:

*Syzygium cordatum* Hoscht. ex C.Krauss is widely used by traditional medicine practitioners to treat many ailments including diarrhoea and diabetes. Despite the folklore use, little evidence can be found in literature to corroborate the claims of therapeutic success of the plant species. The objective of the study was to investigate the antidiarrhoeal and antidiabetic activities of the leaf aqueous extract of the plant species in mice and rats, respectively. The antidiarrhoeal activity of the leaf aqueous extract of *S. cordatum* was investigated using castor oil-induced diarrhoeal test. The antidiabetic activity of the plant extract was studied using streptozotocin-induced diabetes in rats. Acute toxicity study of plant extract was also carried out using a standard method. Leaf aqueous extract of *S. cordatum* significantly reduced the number of diarrhoeal episodes, decreased the stool mass and delayed the onset of castor oil-induced diarrhoea in mice. Loperamide was shown to protect the animals against castor oil-induced diarrhoea. Both the leaf aqueous extract of *S. cordatum* and chlorpropamide significantly lowered the blood glucose levels in both normal and streptozotocin-induced diabetic rats. The LD<sub>50</sub> value obtained for the plant extract was over 4000 mg kg<sup>-1</sup> orally. The results obtained suggest that the leaf aqueous extract of *S. cordatum* has both antidiarrhoeal and antidiabetic activities. This justifies the folklore use of the plant species by traditional medicine practitioners to treat diarrhoea and diabetes. The relatively high LD<sub>50</sub> value obtained for the leaf aqueous extract shows that the plant species is non toxic to mice.

### Introduction

The use of medicinal plants in South Africa is an age long practice. Many people living, especially in rural communities, in the country depend on plant medicines for their healthcare needs. *Syzygium cordatum* Hoscht. ex C. Krauss is one of such plants used by traditional medicine practitioners in South Africa to treat various ailments (Van Wyk *et al.*, 1997; Watt and Breyer-Brandwijk, 1962). *S. cardatum* (family, Myrtaceae) is a mediwn sized tree which can grow as tall as 15 m in height with bluish-green coloured broad leaves. It is widely distributed in the eastern and northeastern parts of South Africa and is known locally as "montlho" in Northern Sotho, "waterbessie" in

Afrikaans and "wndoni" in both Xhosa and Zulu. It is known in English as water berry. All parts of the plant species including the leaves, bark and roots, have been shown to have medicinal properties. Infusion and decoctions of *S. cordatum* have been used by traditional medicine practitioners to treat diarrhoea, stomach aches, diabetes, respiratory problems and tuberculosis and have also been used to induce vomiting (Van Wyk *et al.*, 1997; Hutchings, 1996; Watt and Breyer-Brandwijk, 1962). According to literature, some work has been done on the chemical constituents of *S. cordatum*. Candy *et al.* (1968) showed that the wood and bark of the plant species contain certain triterpenoids and various gallic acids (tannins). Chalannavir *et al.* (2011) and Chisowa *et al.* (1998) investigated the chemical constituents of the essential oil from the leaves of the plant species. However, little work has been done on the pharmacological actions of *S. cordatum* (Musabayane *et al.*, 2005) to corroborate its therapeutic success in the treatment of various ailments as claimed by the traditional medicine practitioners. The main aim of this study was therefore, to investigate the antidiarrhoeal and antidiabetic activities of the leaf aqueous extract of *S. cordatum*. Phytochemical qualitative analysis, acute toxicity and HPLC studies of the plant species were also carried out.

## **Materials and methods**

**Plant materials:** Fresh leaves of *Syzygium cordatum* were collected from Kirstenbosch National Botanical Gardens, Cape Town, South Africa in September 2009. The identification of the plant species was done by both the curator of the Gardens and M. Franz Weitz, a taxonomist in the Department of Biodiversity and Consenrative Biology, University of the Western Cape and a voucher specimen (SC8231) was deposited in the Herbarium of the University.

### **Preparation of leaf aqueous extract of**

***Syzygium cordatum*:** The fresh leaves of the plant species were weighed (1.4 kg), washed with distilled water and dried at 35°C for 4 days. The dried leaves (857 g) were ground to fine powder. Weighed quantity (80 g) of the fine powder was refluxed in 1 l of boiling water, allowed to cool and filtered. The filtrate was then frozen at -80°C and freeze-dried for 5 days. A yield of 19.57 g of dried leaf aqueous extract was obtained and stored in a dessicator for future use. Fresh extract solutions were prepared every day of the experiment by dissolving weighed quantities of the extract in physiological saline. The solutions were administered orally to mice or rats in a volume of 1 mL 100 g of animal using bulbed steel needle.

**Drugs and chemicals:** Castor oil (GR Pharmaceuticals (Pty) Ltd, Atlantis, South Africa) was administered orally in a constant volwne to mice throughout the experiments using bulbed steel needle. Lopermaide ( 4[p-chlorophenyl] -4-hydroxy-N ,N -dimethyl-dipheny 1-1-piperidinebutyramine) hydrochloride (Sigma Chemical Co.,) was dissolved in a minimum amount of 10% ethanol (Merck (Pty) Ltd. and made up to the appropriate volume with physiological saline. Activated charcoal (Sigma Chemical Co.), an aqueous suspension of 5% charcoal and 5% acacia, was prepared. Both the loperamide solution and the charcoal meal were given orally to mice in a volwne of 1 mL 100 g of animal using a bulbed steel needle.

Streptozotocin (STZ, Sigma Chemical Co.) was dissolved in a small volume of 0.1 M citrate buffer pH 4.5, and made up to the appropriate volume with physiological saline, and administered intraperitoneally to rats in a volume of 1 mL 100 g animals. Chlorpropamide (Sigma Co.) was dissolved in physiological saline and administered orally to rats in a volume of 1 mL 100 g of animal using a bulbed steel needle.

**Animals:** Male albino mice bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, South Africa and weighing 18 - 30 g were used for the antidiarrhoeal activity and the acute toxicity testing of the plant species. Y otmg adult male Wis tar rats, bought from the University of Cape Town, South Africa and weighing 160-210 g were used for the antidiabetic activity. The animals were housed in a quiet laboratory with an ambient temperature of 20-24°C and a 12 h light/12-h dark cycle was maintained. They were all maintained on a standard pellet diet and water *ad libitum*. All the animals were fasted for 16 h during which they had free access to water prior to the commencement of the experiments.

**Phytochemical qualitative analysis:** The methods of Ikhiri *et al.* (1992) and Harbome (1984) were used to analyse the dried powdered leaf of *S. cordatum* for various chemical compounds.

**Assessment of antidiarrhoeal activity:** Male albino mice, weighing between 18 and 30 g, were used in groups of six per dose of plant extract or loperamide, a standard antidiarrhoeal drug, throughout the experiments after fasting for 16 h. The method described by (Williamson *et al.*, 1996a) was modified and used to assess the antidiarrhoeal activity of the plant extract. Castor oil, a laxative, known to cause diarrhoea within 4 h, was used to induce diarrhoea in a control group of mice pre-treated with 0.3 mL (p.o.) of physiological saline for 15 min prior to the oral administration of 0.7 mL of castor oil. The onset of diarrhoea, the nwnber of diarrhoeal episodes, stool mass and the nwnber of mice exhibiting diarrhoea were obtained over a 5 h period of obsenration. Experiments were repeated with other groups of animals (test groups), pre-treated for 15 min with either leaf aqueous extract (3.125-50 mg kg<sup>-1</sup>) of the plant species or the standard antidiarrhoeal drug, loperamide (20 mg kg<sup>-1</sup>), both given orally in a volwne of 1 mL 100 g of animals prior to the administration of 0.7 mL (p.o.) of castor oil. The ability of the plant extract to reduce the number of animals exhibiting diarrhoea and/or the number of diarrhoeal episodes is taken as an antidiarrhoeal activity (Williamson *et al.*, 1996a). The doses and pretreatment times used were obtained from preliminary studies in the laboratory. All experiments were carried out between 08:00 hand 17:00 h in a quiet laboratory with an ambient temperature of 20-24°C.

**Assessment of gastrointestinal propulsion of charcoal meal:** The methods used to assess the effect of the plant extract on the gastrointestinal transit of charcoal meal were those described by Williamson *et al.* (1996a) and Kitano *et al.* (1994). Animals were used in groups of six per dose of leaf aqueous plant extract (3.125-50 mg kg<sup>-1</sup>, p.o.) or loperamide (20 mg kg<sup>-1</sup>, p.o.), a standard antidiarrhoeal drug, after fasting for 16 h. Control group was pretreated with 0.3 mL of physiological saline given orally, for 20 min and

then given 0.4 mL of charcoal meal (aqueous suspension of 5% charcoal and 5% gwn acacia) orally. The animals were then killed by ether inhalation 20 min after the administration of charcoal meal and the intestine was removed from the cardia to the rectal end. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Experiments were repeated with other groups of animals (test groups) pretreated with the leaf aqueous plant extract (3.125-50 mg kg<sup>-1</sup>) or loperamide (20 mg kg<sup>-1</sup>), both given orally in a volume of 1 mL 100 g of animals, prior to the administration of 0.4 mL of charcoal meal. All experiments were carried out between 08:00 h and 17:00 h in a quiet laboratory with an ambient temperature of 20-24°C.

**Assessment of castor oil-induced intestinal fluid** accumulation: The methods of Robert *et al.* (1976) and Di Carlo *et al.* (1994) were modified and used to assess the effect of the plant extract on castor oil-induced intestinal fluid accumulation. The animals were used in groups of six per dose of leaf aqueous plant extract (3.125-50 mg kg<sup>-1</sup>, p.o.) or loperamide (20 mg kg<sup>-1</sup>, p.o.), a standard antidiarrhoeal drug, after fasting for 16 hours. The control group of six mice was pretreated with 0.3 mL (p.o.) of physiological saline for 15 min and then given 1.5 mL of castor oil orally. The animals were then killed by ether inhalation 20 minutes after the administration of castor oil and the intestines removed from the pylorus to the caecum. The intestinal contents were then evacuated into a measuring cylinder and the volume measured. Experiments were repeated with other groups of animals pretreated for 15 min with the leaf aqueous plant extract (3.125-50 mg kg<sup>-1</sup>) or loperamide (20 mg kg<sup>-1</sup>), a standard antidiarrhoeal drug, both given orally in a volume of 1 mL 100 g of animals, prior to the oral administration of 1.5 mL of castor oil. All experiments were carried out between 08:00 h and 17:00 h in a quiet laboratory with an ambient temperature of 20-24°C.

**Assessment of antidiabetic activity:** Modified methods of Williamson *et al.* (1996b) and Joy and Kuttan (1999) were used to assess the antidiabetic activity of the leaf aqueous extract of *Syzygium cordatum*. Rats in groups of six each were divided into normoglycaemic and diabetic rats. The normoglycaemic rats were further divided into 'control and test' groups. The control group of six rats received 0.3 mL of physiological saline orally while five groups (of six rats each) of test rats were treated with the leaf aqueous extract of *Syzygium cordatum* (3.125-50 mg kg<sup>-1</sup>, p.o.). One group of six test rats received chlorpropamide (250 mg kg<sup>-1</sup>, p.o.), a standard antidiabetic drug.

Similarly, the diabetic rats were divided further into 'control and test' groups. Diabetes was induced in all the diabetic rats by intraperitoneal injections of streptozotocin (STZ, 90 mg kg<sup>-1</sup>). Following the injection, diabetes was allowed to develop and stabilize in the rats over a period of 3-5 days. The diabetic control group received 0.3 mL of physiological saline orally. Five groups of test diabetic rats received the leaf aqueous extract of the plant species (3.125-50 mg kg<sup>-1</sup>,

p.o.) whereas one group of test diabetic rats received chlorpropamide (250 mg kg<sup>-1</sup>, p.o.). In both normoglycaemic and diabetic rats, blood glucose levels were measured first without treatment (0 h) and then, the animals received either 0.3 mL (p.o.) of physiological saline, plant extract (3.125-50 mg kg<sup>-1</sup>, p.o.) or chlorpropamide (250 mg kg<sup>-1</sup>, p.o.) after which the blood glucose levels were measured at intervals of 1 h, 2 h and 4 h in each treatment group. The blood samples for the measurement of the glucose levels were obtained by pricking the rat tail tip vein. A drop of blood was placed on a glucose test strip and the glucose level read using a compatible Glucometer (Accu-Check Abbot laboratory). Blood was obtained from both normoglycaemic and diabetic rats before treatment (0 h) and then 1, 2 and 4 h after treatment with physiological saline, plant extract or chlorpropamide. Each glucose test strip was used only for one reading. Fasted streptozotocin-treated rats with blood glucose level greater than or equal to 18 mmol L<sup>-1</sup> were considered diabetic. The ability of the plant extract to significantly lower the blood glucose level below 18 mmol L<sup>-1</sup> is taken as an antidiabetic activity (Williamson *et al.*, 1996b; Joy and Kuttan, 1999).

**Acute toxicity testing:** Male albino mice were used in groups of six per dose of plant extract after fasting for 16 h. The method of Lorke (1983) was modified and used to assess the acute toxicity of the leaf aqueous extract of *Syzygium cordatum*. The plant extract was administered orally to mice in graded doses (200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg kg<sup>-1</sup>). Another group of six mice used as control, received 0.3 mL physiological saline orally. Both the test and control animals were then allowed access to food and water and observed over a period of 5 days for any deaths or acute toxicity symptoms such as hypoactivity, piloerection, salivation and so on. The log dose/response (% death) curve plot is used to calculate the median lethal dose (LD<sub>50</sub>) (Amabeoku and Kinyua, 2010). Since no death occurred in all the doses of *R. raveolens* administered, the LD<sub>50</sub> of the plant species was estimated from the data obtained.

### HPLC analysis

**Chromatographic system:** Beckman HPLC system consisting of double pump Programmable Solvent Module model 126; Diode Array detector Module model 168; Samstmg computer 386 with management System Gold (Gold V601) software supplied by Beckman; Column, Cl 8 Bondapak 5 µm and dimensions (250x4.6 mm).

**Chromatographic conditions:** Mobile phase: solvent A: 1% acetic acid, solvent B; methanol, Mode: gradient, flow rate, 1 min/min, injection volume: 10 µL, detector: IN at 350 nm, The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 20%; 5 min, solvent B: 40%, 15 min, solvent B: 60%, 20 min, solvent B: 80% and 27 min, solvent B: 20%. The run rate was 30 min.

**Statistical analysis:** The data obtained from the antidiarrhoeal, antipropulsive or antidiabetic activity and intestinal fluid accwnulation experiments were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison (GraphPad Prism, version 5.0, GraphPad Software, Inc., San Diego CA 92130, USA) and presented as mean±Standard Error Mean (SEM). However, the data on the number of animals exhibiting diarrhoea were analysed using the Chi square test. In the above cases, p-values of less than 5% ( $p < 0.05$ ), 10 were considered to be significant.

**Ethics clearance:** The Ethics Committee of the University of the Western Cape approved the experimental protocol (07/04/31) used in the present study and this conforms to the "Guide to the care and use of animals in research and teaching" of the University.

## Results

**Effect of leaf aqueous extract of *Sy zygium cordatum* on castor-oil induced diarrhoea:** Castor-oil (0.7 mL) given orally, produced diarrhoea within 4 h in all the six animals used and also produced a considerable amount of stool. *Syzygium cordatum* (3.125-50 mg kg<sup>-1</sup>, p.o.) significantly reduced the faecal output produced by castor oil. *S. cordatum* in all the doses used, reduced the faecal output from 100% produced by castor-oil to between 40 and 61 %. *S. cordatum* (6.25-50 mg kg<sup>-1</sup>, p.o.) significantly and in a dose dependent manner, delayed the onset of castor oil (0.7 mL, p.o.)-induced diarrhoea. Doses of 3.125-50 mg kg<sup>-1</sup> (p.o.) significantly reduced castor oil (0.7 mL, p.o.)-induced diarrhoeal episodes but did not significantly alter the nwnber of animals exhibiting diarrhoea. Loperarnide (20 mg kg<sup>-1</sup>, p.o.) significantly reduced the faecal output produced by castor-oil (0.7 mL, p.o.) from 100% produced by castor oil to 8.7%. The dose of 20 mg kg<sup>-1</sup> (p.o.) of loperarnide profoundly delayed the onset of diarrhoea produced by castor-oil (0.7 mL, p.o.) and profoundly reduced the incidence of castor-oil (0.7 mL, p.o.)-induced diarrhoea by protecting 83% of animals against the diarrhoea. Loperamide (20 mg kg<sup>-1</sup>, p.o.) profoundly reduced the nwnber of castor-oil (0.7 mL, p.o.)-induced diarrhoeal episodes by 96% (Table !).

**Effect of leaf aqueous extract of *Sy zygium cordatum* on gastrointestinal transit of charcoal meal:** The mean length of intestine travelled by 0.4 mL (p.o.) of charcoal meal in control mice pretreated with 0.3 mL (p.o.) of physiological saline was 87.73±5.01 %. Leaf aqueous extract of *Syzygium cordatum* (6.25-50 mg kg<sup>-1</sup>, p.o.) significantly and dose dependently decreased the mean length of intestine travelled by 0.4 mL (p.o.) of charcoal mean. The propulsion of the charcoal mean was inhibited by 21.25-50.93% at doses of 3.125-50 mg kg<sup>-1</sup> (p.o.) of *S. cordatum*. Loperarnide (20 mg kg<sup>-1</sup>, p.o.) significantly reduced the mean length of intestine travelled by charcoal meal (0.4 mL, p.o.). Loperamide (20 mg kg<sup>-1</sup>, p.o.) inhibited the propulsion of charcoal meal by 79.06% (Table 2).

Table 1: Effect of leaf aqueous extract of *Syzygium cordatum* on castor oil-induced diarrhoea in mice

Treatment groups (mg kg <sup>-1</sup> )	Mass of stool (g) (Mean±SEM)	Faecal output (%)	Onset of diarrhoea (min) (Mean±SEM)	No. of diarrhoeal episode (Mean±SEM)	Percentage diarrhoeal episode inhibition (%)	No. of animals exhibiting diarrhoea
<b>PS</b>						
0.3 mL	3.67±0.29	100.0	22.17±2.10	25.17±0.93		6/6
<b><i>Syzygium cordatum</i></b>						
3.125	2.22±0.14**	60.5	42.17±3.90	10.83±1.49***	56.97	6/6
6.25	2.22±0.39**	60.5	70.83±5.67 <sup>b</sup>	12.17±2.40***	51.65	6/6
12.50	1.80±0.27***	49.0	71.50±4.32 <sup>b</sup>	8.00±1.05***	68.22	6/6
25.00	1.45±0.28***	39.5	79.33±5.97 <sup>b</sup>	5.67±1.30***	77.47	6/6
50.00	1.83±0.21***	49.9	105.17±19.60***	9.83±0.96***	60.95	6/6
<b>Loperamide</b>						
20.00	0.32±0.18***	8.7	119.83±16.57***	1.00±0.00***	96.02	1/6 <sup>c</sup>

\*p<0.01, \*\*p<0.005, \*\*\*p<0.001 vs. castor oil (0.7 mL, p.o.) control. ANOVA (n = 6), <sup>b</sup>p<0.005 vs. castor oil (0.7 mL, p.o.) control. Chi-squared test (n = 6), PS: Physiological saline, %: Percentage

**Effect of leaf aqueous extract of *Syzygium cordatum* on castor oil-induced intestinal fluid accumulation:** The mean intestinal fluid volume produced by castor oil (1.5 mL, p.o.) in control animals pretreated with physiological saline (0.3 mL, p.o.) was 1.42±0.19 mL. *Syzygium cordatum* (3.125-50 mg kg<sup>-1</sup>, p.o.) significantly reduced the mean intestinal fluid volume produced by castor oil (1.5 mL, p.o.). The mean intestinal fluid volume was reduced or inhibited by 41.55-59.15%. Loperamide (20 mg kg<sup>-1</sup>, p.o.) significantly reduced the mean intestinal fluid volume produced by castor oil (1.5 mL, p.o.). The mean intestinal fluid volume produced by castor oil (1.5 mL, p.o.) was inhibited by loperamide (20 mg kg<sup>-1</sup>, p.o.) by 52.82% (Table 3).

**Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations (mmol L<sup>-1</sup> of normoglycaemic rats:** Physiological saline (0.3 L, p.o.) did not significantly alter the blood glucose concentration of fasted normal rats throughout the 4 h period of observation. *Syzygium cordatum* (3.125-6.25 mg kg<sup>-1</sup>, p.o.) did not significantly affect the blood glucose concentration of fasted normal rats throughout the 4 h of observation. Doses of 12.50-50 mg kg<sup>-1</sup> (p.o.) of *Syzygium cordatum* significantly reduced the blood glucose concentration of fasted normal rats from the second to the fourth hour of observation with a percentage maximal reduction of 28.60-32.79%. Chlorpropamide (250 mg kg<sup>-1</sup>, p.o.) significantly reduced the blood glucose concentration of fasted normal rats from the first hour through to the fourth hour of observation and the percentage maximal reduction was 43.26% (Table 4).

Table 2: Effect of leaf aqueous extract of *Syzygium cordatum* on gastrointestinal transit of charcoal meal in mice

Treatment groups (mg kg <sup>-1</sup> )	Length of intestine travelled (%) Mean ± SEM	Percentage inhibition in length of intestine travelled (%)
<b>PS</b>		
0.3 mL	87.73±5.01	
<b><i>Syzygium cordatum</i></b>		
3.125	69.09±4.87	21.25
6.25	63.17±4.57*	28.00
12.50	62.34±7.20*	28.94
25.00	58.48±5.95**	33.34
50.00	43.05±9.84***	50.93
<b>Loperamide</b>		
20.00	18.37±3.14***	79.06

\*p<0.05, \*\*p<0.005, \*\*\*p<0.001 vs. charcoal meal (0.4 mL, p.o.) control. ANOVA (n = 6), PS: Physiological saline, %: Percentage

Table 3: Effect of leaf aqueous extract of *Syzygium cordatum* on castor-oil induced intestinal fluid accumulation in mice

Treatment groups (mg kg <sup>-1</sup> )	Intestinal fluid volume (mL) (Mean±SEM)	Percentage inhibition (%)
<b>PS</b>		
0.3 mL	1.42±0.19	
<b><i>Syzygium cordatum</i></b>		
3.125	0.58±0.04**	59.15
6.25	0.83±0.15*	41.55
12.50	0.80±0.02*	43.66
25.00	0.80±0.05*	43.66
50.00	0.73±0.05*	48.59
<b>Loperamide</b>		
20.00	0.67±0.07**	52.82

\*p<0.01, \*\*p<0.001 vs. castor oil (1.5 mL, p.o.) control. ANOVA (n = 6). PS: Physiological saline, %: Percentage

Table 4: Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations of normoglycaemic (normal) rats

Treatment groups (mg kg <sup>-1</sup> )	Glucose concentration (mmol L <sup>-1</sup> )				Maximal reduction	Percentage maximal reduction (%)
	Before treatment		After treatment			
	0 h	1 h	2 h	4 h		
<b>PS</b>						
0.3 mL	6.18±0.24	5.82±0.20	5.65±0.18	6.10±0.20	0.53	8.58
<b><i>Syzygium cordatum</i></b>						
3.125	6.67±0.21	5.90±0.13	5.57±0.14	5.72±0.27	1.10	16.49
6.25	6.75±0.25	6.03±0.21	5.37±0.18	5.80±0.24	1.38	20.44
12.50	6.33±0.56	5.98±0.22	4.73±0.32*	4.52±0.38**	1.81	28.60
25.00	6.80±0.20	5.92±0.32	4.75±0.15*	4.60±0.20**	2.20	32.35
50.00	6.80±0.29	5.58±0.29	4.62±0.12*	4.57±0.42**	2.23	32.79
<b>Chlorpropamide</b>						
250	6.75±0.40	4.85±0.29*	3.83±0.37***	3.87±0.35***	2.92	43.26

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. physiological saline (0.3 mL, p.o.) control, ANOVA (n = 6), Values are expressed as Mean±SEM, PS: Physiological saline, %: Percentage

Table 5: Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations of streptozotocin-treated diabetic rats

Treatment groups (mg kg <sup>-1</sup> )	Blood glucose concentration (mmol L <sup>-1</sup> )				Maximal reduction	Percentage maximal reduction (%)
	Before treatment		After treatment			
	0 h	1 h	2 h	4 h		
<b>PS</b>						
0.3 mL	21.40±1.02	23.67±1.26	23.67±0.89	23.88±1.00		
<b><i>Syzygium cordatum</i></b>						
3.125	23.37±1.10	23.78±0.66	23.20±0.60	22.95±0.47	0.83	3.49
6.25	22.68±1.04	22.43±0.52	20.77±0.55	17.38±1.11*	5.30	23.37
12.50	20.32±0.59	21.28±1.14	15.25±0.78*	14.22±1.80*	7.06	33.18
25.00	20.17±0.57	21.41±1.53	14.12±0.12*	13.80±0.93*	7.61	35.54
50.00	20.53±2.08	14.42±0.87*	14.07±0.68*	13.68±0.89*	6.85	33.37
<b>Chlorpropamide</b>						
250	23.23±0.94	15.55±1.34*	6.38±1.12*	4.05±0.24*	19.18	93.42

\*p<0.001 vs streptozotocin (90 mg kg<sup>-1</sup>, i.p.) control, ANOVA (n = 6), Values are expressed as Mean±SEM, PS: Physiological saline, %: Percentage

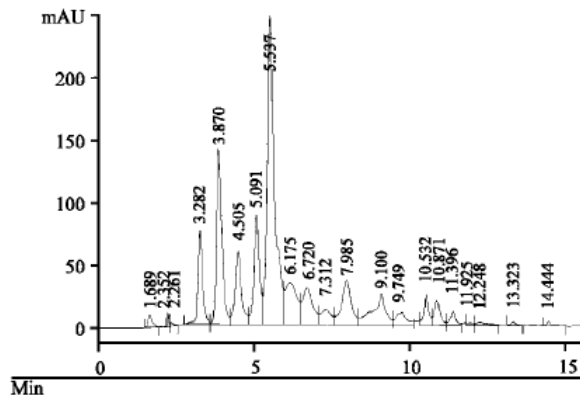


Fig. 1: Chromatographic fingerprint of *Syzygium cordatum*



Effect of leaf aqueous extract of *Syzygium cordatum* on blood glucose concentrations ( $\text{mmol L}^{-1}$ ) of streptozotocin-treated diabetic rats: Streptozotocin (90 mg, p.o.) raised the blood glucose concentration of fasted animals to  $21.40 \pm 1.02 \text{ mmol L}^{-1}$ . Physiological saline (0.3 mL, p.o.) did not significantly affect the blood glucose concentration of fasted diabetic rats throughout the 4 h of observation. Dose of 3.125 mg (p.o.) of *Syzygium cordatum* did not significantly alter the blood glucose concentration of the fasted diabetic rats throughout the 4 h of observation. *Syzygium cordatum* (6.25  $\text{mg kg}^{-1}$  p.o.) significantly reduced the blood glucose of the fasted diabetic rats in the 4th h of observation with a maximal reduction of 23.37%. *Syzygium cordatum* (12.50-25 mg, p.o.) significantly reduced the blood concentration of fasted diabetic rats from the 2nd to the 4th h of observation with a percentage maximal reduction of 33.18- 35.54%. At a dose of 50  $\text{mg kg}^{-1}$  (p.o.), *S. cordatum* significantly reduced the blood glucose concentration of fasted diabetic rats from the 1st h through to the 4th h of observation with a percentage maximal reduction of 33.37%. Chlorpropamide (250  $\text{mg kg}^{-1}$ , p.o.) profoundly reduced the blood glucose concentration of fasted diabetic rats from the 1st hour through to the 4th hour of observation with a percentage maximal reduction of 93.42% (Table Acute toxicity test: The leaf aqueous extract of *Syzygium cordatum* (200, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000  $\text{mg kg}^{-1}$  administered orally to mice did not cause any death to or any acute toxicity symptoms in the animals in all the doses used. The highest dose tested being 4000  $\text{mg kg}^{-1}$  should be the NO-Adverse-Effect-Level (NOAEL). The  $\text{LD}_{50}$  value for the plant species may, therefore, be greater than 4000  $\text{mg kg}^{-1}$  (p.o.).

Phytochemical analysis: The phytochemical qualitative of the analysis of the plant species showed the presence of tannins, saponins, alkaloids, flavonoids, triterpene steroids and reducing sugars.

HPLC analysis: The chromatographic fingerprint of the leaf methanol extract of *S. cordatum* showed major peaks at the following retention times (minutes): 3.282, 3.870, 5.537 and 7.985 (Fig. 1)

## Discussion

In this study, the plant extract, up to the highest dose (4000  $\text{mg kg}^{-1}$ , p.o.) used in the acute toxicity test, did not cause any death to or acute toxicity symptoms in the mice. The  $\text{LD}_{50}$ , therefore, may be greater than 4000  $\text{mg kg}^{-1}$  (p.o.). This relatively high  $\text{LD}_{50}$  shows that the plant extract is non-toxic and/or safe in mice. Traditional medicine practitioners are known to use the plant for treatment in the form of infusion (Van Wyk *et al.*, 1997). The HPLC chromatogram of *S. cordatum* showed characteristic peaks at a wavelength of 350 nm. The pharmacological screening results obtained in the present study, show that *S. cordatum* (3.125-50  $\text{mg kg}^{-1}$ , p.o.) antagonized the diarrhoea

produced by castor oil (0.7 mL, p.o.). Loperamide (20 mg kg<sup>-1</sup> p.o.) also antagonized the castor oil-induced diarrhoea in mice. Castor oil, an irritant laxative, is thought to produce diarrhoea by being hydrolysed in the upper small intestine to ricinoleic acid which exerts its effects by irritating the mucosa of the gastrointestinal tract, resulting in an increase in intestinal motility (Altman, 2001). Furthermore, ricinoleic acid has been shown to diminish the permeability of sodium and chloride ions and also stimulate the release of prostaglandin, known to cause diarrhoea (Tunaru *et al.*, 2012; Zavala *et al.*, 1998). In addition, the works of Capasso *et al.* (1994) and Mascolo *et al.* (1994), on the effect of NG-nitro-L-arginine methyl ester, an inhibitor of Nitric Oxide (NO) synthase, on the dissociation of castor oil-induced diarrhoea and mucosal injury in rat, showed that nitric oxide may mediate castor oil-induced diarrhoea. Loperamide, an opioid derivative and a standard antidiarrhoeal drug, is thought to decrease intestinal motility by binding to mu receptors on neurons in the submucosal neural plexus of the intestinal wall. This leads to the segmental contractions in the colon increasing, the propulsive movement of the small intestine and colon being inhibited and the transit time of the intestinal content being prolonged (Altman, 2001). Loperamide is also known to have an antimuscarinic activity contributing to the inhibition of peristalsis by inhibiting contractions in both the longitudinal and circular muscles (Waller *et al.*, 2005a; Camillen *et al.*, 2002; Altman, 2001). In this study, therefore, loperamide may be antagonizing castor oil-induced diarrhoea by decreasing the intestinal motility. Similarly, *Syzygium cordatum* may be said to exert its antidiarrhoeal activity by slowing intestinal motility.

In the present study, the leaf aqueous extract of *S. cordatum* significantly antagonized the gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced intraluminal accumulation of fluid volume. Similarly, loperamide significantly antagonized the gastrointestinal transit of charcoal meal and also significantly reduced the castor oil-induced intraluminal accumulation of fluid volume. According to Di Carlo *et al.* (1994), agents that reduce intestinal motility and secretion may possess antidiarrhoeal activity. Furthermore, Nwafor *et al.* (2000) have shown that agents that suppress intestinal fluid accumulation may inhibit gastrointestinal functions. The above reports lend support to the suggestion that *S. cordatum* may be exerting its antidiarrhoeal activity by slowing intestinal motility. It is also pertinent to state that the data obtained on the antidiarrhoeal activity of *S. cordatum* is in agreement with that of Sibandze *et al.* (2010) who reported the antidiarrhoeal properties of *Breonadia salicina*, *Syzygium cordatum* and *Ozoroa sphaerocarpa* against a diarrhoea-causing pathogen, *Escherichia coli*. Candy *et al.* (1968) in their study have shown that *S. cordatum* contain certain triterpenoids and various gallic acids (tannins). The qualitative phytochemical analysis of the powdered leaf of *S. cordatum* carried out in this study showed that the plant species contains tannins, saponins, alkaloids, triterpene steroids, flavonoids and reducing sugars. Several studies have shown tannins to have antidiarrhoeal activity. Tannin containing drugs have been used for the treatment of diarrhoea and other related disorders (Bruneton, 1999; Frei *et al.*, 1998). Astringents

such as tannins have been known since the last century to have antisecretory effect in the gastrointestinal tract and have been used to treat diarrhoea (Farthing, 2000). It is probable therefore, that the presence of tannins in the plant species as shown by Candy *et al.* (1968) and the present study, may contribute to the antidiarrhoeal activity of *S. cordatum*.

*Syzygium cordatum* has been shown in the present study to have an antidiabetic activity. Chlorpropamide, a sulphonylurea, and an oral antidiabetic agent used for the treatment of Type 2 or Non-insulin Dependent Diabetes (NIDD), was used as a standard drug in this study. It is thought to act by stimulating and increasing the release of endogenous insulin from the pancreatic beta cells of the Islet of Langerhans (Rang *et al.*, 2008; Waller *et al.*, 2005b). Streptozotocin (STZ) used to induce diabetes in this study, on the other hand, is thought to produce diabetes by a rapid depletion of pancreatic beta cells and thereby, reducing insulin release and causing hyperglycaemia (Mahomed and Ojewole, 2003). Thus, the STZ-induced rat diabetes model has the hallmark of non- insulin dependent diabetes or Type 2 diabetes. In this study, *S. cordatum* (12.5-50 mg kg<sup>-1</sup>, p.o.) and chlorpropamide (250 mg kg<sup>-1</sup>, p.o.) significantly reduced the blood glucose concentration of fasted normal rats while 0.3 mL (p.o.) of physiological saline did not alter the blood glucose concentration of the fasted normal rats. Similarly, both the leaf aqueous extract of *S. cordatum* (12.5-50 mg kg<sup>-1</sup>, p.o.) and chlorpropamide (250 mg kg<sup>-1</sup>, p.o.) significantly reduced the glucose concentration of diabetic rats treated with streptozotocin. Since chlorpropamide used to treat diabetes, acts by stimulating insulin secretion from pancreatic beta cells and also promoting peripheral glucose uptake and utilization (Rang *et al.*, 2008; Waller *et al.*, 2005b), it is probable that *S. cordatum*, may be acting in a similar manner. The result obtained in this study is in agreement with the study of Musabayane *et al.* (2005) who showed that *S. cordatum* leaf extract significantly lowered the plasma glucose and hepatic glycogen levels in STZ-induced diabetic rats.

In this study, the phytochemical analysis carried out revealed the presence of alkaloids and flavonoids amongst other chemical metabolites in *S. cordatum*. Punitha *et al.* (2005) in their study on berberine, an alkaloid, and antidiabetic activity, showed that alkaloids have antidiabetic activity. Dineshkumar *et al.* (2010) in their study on the antidiabetic and hypolipidemic effects of mahanimbin from *Murraya koenigii* leaves also showed that alkaloids have antidiabetic activity. Flavonoids have also been shown by the study of Fawzy *et al.* (2008) which investigated the antidiabetic and antioxidant activities of major flavonoids of *Cynanchum acutum* L., to have antidiabetic activity. Furthermore, Hule *et al.* (2011) in their study on the evaluation of the antidiabetic effects of *Elaeocarpus ganitrus* in experimental animals, showed that alkaloids and flavonoids have antidiabetic activities. It is probable therefore, that the flavonoids and alkaloids found in *S. cordatum* may be contributing to its antidiabetic activity.

## **Conclusion**

The data obtained in this study indicated that *Syzygium cordatum* have both antidiarrhoeal and antidiabetic activities. This project was not set out to investigate the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum*. However, various studies as indicated above have shown that castor oil- induced diarrhoea may also involve increase in electrolyte permeability, increased release of prostaglandins and nitric acid mechanism. It is probable therefore, that the antidiarrhoeal activity of *S. cordatum* may involve the inhibition of electrolyte permeability, prostaglandin release and nitric acid mechanism. The antidiabetic activity may be due to the plant species stimulating the release of insulin from the pancreatic beta cells since streptozotocin used to induce diabetes is known to act by rapidly depleting pancreatic beta cells and thus, reducing insulin release. Tannins and alkaloids and flavonoids may also be contributing to the antidiarrhoeal and antidiabetic activities of *S. cordatum*, respectively. These data may justify the use of the plant species by traditional medicine practitioners in the treatment of diarrhoea and diabetes. However, further studies on the acute toxicity, and the mechanisms of the antidiarrhoeal and antidiabetic activities of *S. cordatum* need to be carried out to enhance the safety and efficacy of the plant species.

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